Phytochemical Screening And Test For Reducing Uric Acid Levels In Male Rats After Administration Of Ethanol Extract Of The Herb Binara (*Artemisia vulgaris* L.)

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Abstract.

Hyperuricemia is a metabolic disease generally characterized by high uric acid levels in the blood. The general treatment strategy for hyperuricemia is to decrease uric acid production using xanthine oxidation reductase inhibitors such as allopurinol and uricosuric agents. However, the drugs showed side effects, including skin rashes, diarrhoea and liver damage. To overcome the side effects of synthetic drugs, alternatives are used, namely by researching to find plants that have activity in reducing uric acid levels. The activity of reducing uric acid levels in plants is likely due to the presence of flavonoid compounds as antioxidants. Flavonoids active in reducing uric acid levels are kaempferol through xanthine oxidase inhibitors. Quercetin also has the potential to reduce uric acid levels through inhibition of the xanthine oxidase enzyme. Binara plant (Artemisia vulgaris L.) has flavonoids, tannins, alkaloids, glycosides and saponins which have potential as antioxidants and analgesics, anti-inflammatory, immunomodulating, and hepatoprotective, through secondary metabolites of flavonoids, terpenes, and phenolic acids. The Karo people have traditionally used Binara leaves to treat wounds, diarrhoea and heartburn by chewing some of the leaves and then placing them on the wounds outside the body, such as cuts and for diarrhoea and stomach ulcers and placing them around the sick stomach. This study was conducted to know the anti-hyperuricemia effect of the Ethanol Extract of Herba Binara (Artemisia vulgaris L.) on gout rats; phytochemical screening was carried out first to determine the content of Herba Binara (Artemisia vulgaris L.) compounds. The results showed that Herb Binara fulfilled the simplicia characterization requirements and contained secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, saponins and steroids. Herb Binara Ethanol Extract has been shown to reduce uric acid levels in hyperuricemia rats, with the best concentrations being 400 and 800 mg/kg body weight. This was based on significantly different results p>0.05 compared to the positive control group.

***Keywords**: Binara herbs, Artemisia vulgaris L., Gout, and Hyperuricemia.*

I. INTRODUCTION

Hyperuricemia is a metabolic disease generally characterized by high uric acid levels in the blood, and this is a significant risk factor in the pathogenesis of chronic kidney disease, hypertension, gout arthritis and metabolic syndrome. Some previous studies reported clinical symptoms of kidney damage in all hyperuricemia patients [1].General treatment strategies for hyperuricemia decreased gout production using xanthin inhibitors oxidation of reductases such as allopurinol and uricosuric agents. However, the drugs show side effects, including skin rashes, diarrhoea and liver damage [2]. While uricosuric drugs, in some cases reported cause liver toxicity [3]. To overcome the side effects of synthetic drugs, alternatives are used, namely by researching to find plants that have the activity of decreasing uric acid levels. The activity of decreasing uric acid levels in plants is thought to have flavonoid compounds as antioxidants. Flavonoids that actively reduce uric acid levels are kaempferol through Xantin oxidase inhibitors [4]. Quercetin also has the potential to reduce uric acid levels through the inhibition of xanthine oxidase enzymes [5]. Kuersetin and Myricetin are xanthin oxidase inhibitors and have free radical absorbers from the formation of high uric acid because when formed, uric acid is always followed by the formation of 1 superoxide anion molecule radical anion (O2-) and two hydrogen peroxide molecules (H2O2) which can trigger lipids peroxidation so that it can damage the kidneys [6].Binara plant (*Artemisia vulgaris* L.), known as Mugwort from the results of the study, shows that this plant has flavonoid compounds, tannins, alkaloids, glycosides and saponins which have the potential to be antioxidants and analgesics [7].

In addition, Artemisia Vulgaris L. also has the potential to be anti-inflammatory, immunomodulation, and hepatoprotection through secondary metabolites of flavonoids, terpenes, and

phenolic acid [8]. The Karo community has traditionally used Binara leaves to treat wounds, diarrhoea, and heartburn by chewing a few leaves and then affixed to the wound outside the body, such as cut-away wounds and for diarrhoea and stomach mules attached around the affected stomach [9]. Some of the artemisia vulgaris activities L. has several potentials such as antimalarials, antibacterial, antioxidants and antidiabetic and antioxidants [8, 10, 11]. Meliala's research (2018) shows that the total flavonoid level in the EEHB sample is 98.08 MGQE/g sample with an IC50 value of 95.05 ppm in the sense of having moderate to high antioxidant activity. Besides that, it could be an immunomodulator with a carbon clearance method, antibody titer, and leukocyte and differential leukocyte measurements [12, 13]. This study aimed to know the anti-hyperuricemia effect of Binara Herb Ethanol extract (*Artemisia vulgaris* L.). In gout rats, phytochemical screening is done first to determine binary herbal compounds' content (*Artemisia vulgaris* L.).

II. METHODS

Tools and Materials

The tools used in this study include Freeze Dryer Dryer Cabinets (Edwards), Electric Oven (Memmert), Naberrtherm, Water Bangs, Rotary Evaporator (Heidolph), Surgical Equipment, Round Packaging, Animal Balance (Presica) Digital scales (Mettler Tolledon), Rough Balance (Ohaus), Blender (Phillips), Laboratory Glass Devices, Stopwatch, Mortar and Stamfer, Aluminum Foil, Filter Paper, Oral Sonde, 1 ml Spuit (Terumo), Microscopes (Boeco), oral sonde.

The material used in this study is Binara Herb. The chemicals used unless otherwise, stated quality pro-analysis are the reagent of bouchardate, dragendroff, Mayer, iron (III) chloride, molisch, lead (II) acetic acid, chloride acid, methanol, chloroform-isopropanol, ethanol 96%, Liebermann- Bouchard, N-Hexan, Toluene, Chloroform, Magnesium Powder, Zinc Powder, Na-CMC (Sodium Carboxy Methyl Cellulose), Allopurinol, Potassium Oxonate (Sigma-Aldrich), Chloride Acid (HCl) 2n (Merck), Magnesium Powder (Merck), chloroform.

Experimental Animals

This study uses the male Wistar strain with a body weight of 180g ± 10% (165g-200g). First, the mice are acclimatized for two weeks to adjust to their environment. Rats are divided into eight groups; each group consists of 4 specified based on the Federer Formula. From this study, researchers have received approval from the ethics commission with number 0622/KEPH-FMIPA/2021.

Manufacturing Simplicia

Plant materials are taken randomly without comparing them with the same plants from other regions. The sample was obtained from the village of Cinta Rakyat, Kec. Merdeka, Berastagi. An identification of Binara Herba Plant Samples in Herbarium Medanense (Meda) in the Botanical Field of the University of Sumatera Utara. Binara Herba that has been collected is washed thoroughly with running water, drained then sorted wet. This material is then dried in the drying cabinet, sorted, and weighed. The material is then mashed using a blender. Simplisia is put in a plastic container and tied, given etiquette and then stored in a place protected from sunlight [14].

Examination Of Simplicia Characteristics

Check the characteristics of simplicia, including a macroscopic examination of simplistic, microscopic powder symposia, determination of water content, determination of water-soluble juice content, determination of ethanol soluble juice content, determination of total ash content, and determination of non-acid-soluble ash content [14].

Macroscopic Examination And Microscopic Examination

Macroscopic and organoleptic examinations are done by observing the shape, odour, and taste of the Binara Herb and simplisia powder binara. Microscopic examination is carried out on the simplisia powder. Simplisia powder is placed on a glass of object that has been met with a chloraralhydrous solution, covered with a cover glass, and then observed.

Simplisia Phytochemical Screening

Phytochemical Screening of Binara Herba Simplisia powder includes an examination of compounds of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids.

Making Ethanol Ethanol Extract Binara (EEHB)

The manufacture of Binara Herb Ethanol extract is as follows: A total of 500 grams of Binara herb is put into a vessel and then added ethanol solvent until simplicia powder is submerged with ten parts of ethanol solvent, closed and soaked for the first 6 hours, then allowed to stand for 18 hours and separated by Maserati by filtering and repeated the search process three times with half the number of volumes of the solvent in the first search. The concentration of the extract was carried out using a rotary evaporator at a temperature of ± 40^{o}C until a thick extract was obtained [15].

Testing The Effect Of Decreased Uric Acid Levels EEHB

Test animals are grouped into eight groups, each consisting of 4 mice weighed and marked on the tail. Each group measured the level of fasting gout (7-9 hours) on the 0th day, 3, 7, and 14 through urine. Each group is given the following treatment:

a. Group I: Normal Group
b. Group II: Oxonate potassium induction 250 mg/kg BW (days 0-14), then Na-CMC 0.5% and standard feed (days 15-28)
c. Group III: Oxonate Potassium Induction 250 mg/kg BW (Day 0-14)
d. Group IV: Induction of Oxonate Potassium 250 mg/kg BW (Day 0-14) Then EEHB Suspension Dosage 50 mg/kg BW (Day 15-28)
e. Group V: Induction of Oxonate Potassium 250 mg/kg BW (Day 0-14) Then EEHB Suspension Dose 100 mg/kg BW (Day 15-28)
f. Group VI: Oxonate Potassium Induction 250 mg/kg BW (Day 0-14) then EEHB Suspension Doses 200 mg/kg BW (Day 15-28)
g. Group VII: Oxonate Potassium Induction 250 mg/kg BW (Day 0-14) Then EEHB Suspension Dosage 400 mg/kg BW (Day 15-28)
h. Group VIII: Oxonate Potassium Induction 250 mg/kg BW (Day 0-14) Then EEHB Suspension Dosage 800 mg/kg BW (Day 15-28)

On the 28th day, the results of measuring uric acid levels were noted. Rats are said to be hyperuricemia if uric acid levels> 4.37 ± 1.11 mg/dl, and subsequently calculated per cent decreased uric acid levels [16].

III. RESULT AND DISCUSSION

The Results Of A Macroscopic And Microscopic Simplicia Examination

A macroscopic simplicia examination has been carried out on the Binara Herb. The results show that the upper part of the leaf surface is smooth and has a brownish-dark green colour, while the subsurface has a vaginal discharge colour. Flowers have a yellowish-white colour and are small. A microscopic examination has been carried out of simplicia Binara powder. The results show the presence of parenchyma containing red pigments and peripheral parenchyma. Macroscopic and microscopic examinations can be seen in Figure 1.

Fig 1. Macroscopic and microscopic examination of Binara Herb. a) macroscopic, (b) microscopic, 1. Parenchyma contains red pigments, 2. Parenchyma of the edge

The Results Of The Characterization Of Simplicia Binara Herb

Examination of water content, examination of water-soluble levels, examination of soluble juice levels in ethanol, examination of total ash content, and examination of acid-soluble ash content have been carried out. The results are listed in Table 1.

Table 1. Examination Results of the Characterization of Simplisia Herb Binara

Parameter	Result (%)	Requirements (MMI)
Water Content	8.63	<10
Water soluble essence content	19.42	>5.0
Ethanol soluble essence content	5.17	>4.5
Total ash content	12.56	<13
Acid insoluble ash content	0.93	<1.5

The results of determining the water content of the Binara herbal simplicia obtained 8.63%; this follows the standardization of Simplisia water content in general with the condition that it is no more than 10%. If the water content is> 10%, it will damage simplicia because microbes and fungi can grow there [17]. Examining water-soluble juice levels to determine the levels of polar chemical compounds contained in the Binara herbal simplicia results is 19.42%. In contrast, the level of ethanol soluble juice determines the levels of soluble compounds in ethanol; both polar and nonpolar compounds are 5, and 17%. Examination of total and non-acid-soluble ash content is done to ensure that no heavy metal contained in simplicia exceeds the standard because it can be toxic to health. Several inorganic compounds, namely Mg, Ca, Na, Zn, and K, can be known by knowing the level of total ash. Inorganic compounds such as silicate can be known by examining insoluble grey levels in acids. Meanwhile, there are two types of total ash, namely physiological and non -physiological ash content. Physiological ash content is the amount of ash in each plant tissue, while the level of non -physiological ash is the remaining combustion from outside the surface [17]. The determination of total ash content in Binara Simplisia is 12.56% and 0.93% for acid-soluble ash content. The importance of standardization is because every plant has an active ingredient that varies even though the type of plant is the same. Standardization is expected to remain consistent so that the benchmark in testing has balanced, dynamic content.

Results Of Phytochemical Screening

The results of the phytochemical screening of Binara herbs in Table 2 show the presence of alkaloid compounds with the addition of Mayer reagent solution, forming a white-clotting precipitate, with a Bouchardat reagent solution forming blackish brown deposits, and dragendorff reagent solution formed red [15]. Examining flavonoid compounds with magnesium powder and concentrated hydrochloric acid produces a red solution [15]. Molisch reagents and concentrated sulfuric acid form a purple ring [15]. The sample, with heat distilled water and beaten, strongly produced a stable foam; then, HCl 2 N was added, indicating the presence of a group of saponin compounds [15]. The addition of FeCl3 gives a blackish-green colour showing the tannin compounds' presence [15]. Examination of triterpenoid/steroid compounds with adding a few drops of Liebermann-Burchard reagents produces pink or purple, which shows the triterpenoids compound group [18]. Examination of the secondary metabolite compound is closely related to the efficacy of plants in a pharmacological manner. Flavonoids and steroids/triterpenoids contained can be efficacious as antihydeperurisemia drugs. Active flavonoids can stabilize uric acid levels, namely kaempferol, through Xantin oxidase inhibitors [4]. Quercetin also has the potential to stabilize uric acid levels by inhibiting xanthine oxidase enzymes [5].

Table 2. Results of the phytochemical screening of Binara herbs

Secondary Metabolites	Reactants	Result
Alkaloids	Dragendroff	+
	Bouchardat	+
	Meyer	+
Flavonoids	Mg Powder + Amyl Alcohol + HCl_p	+
Glycosides	Molish+H_2SO_4	+
Saponins	Hot/shaken water	+
Tannins	$FeCl_3$	+
Triterpenes/Steroids	Lieberman-Bourchat	+

Uric Acid Levels Before And After Induction Of Oxonate Potassium

Test mice are grouped into eight treatment groups: positive groups (allopurinol), regular, negative, and five doses of treatment (Eehb 50, 100, 200, 400, and 800). All groups were made hyperuricemia by induced potassium oxonate 250 mg/kg body weight for 14 days intraperitoneal, then fasting gout (7-9 hours) on days 0, 3, 7, and 14 were measured. After the rat is induced, the potassium oxonate solution 250 mg/kg BW BW 14 have increased uric acid levels (hyperuricemia)> 4.37 ± 1.11 mg/dl [16]. The results of gout measurements can be seen in Table 3.

Table 3. Measurement Results of Uric Acid Before and After Induced with Oxonate Potassium 250mg/KgBB

Group	Uric Acid Before Induction Levels days-0 (mg/dL)	Uric Acid Levels After Induction of PO ± SEM (mg/dL)		
		Days 3	Days 7	Days 14
Normal Control	0.37 ± 0.09	0.52 ± 0.17	0.80 ± 0.08	0.87 ± 0.09
Negative Control	0.48 ± 0.09	2.57 ± 0.20	4.33 ± 0.22	8.78 ± 1.07
Positive Control	0.35 ± 0.10	2.13 ± 0.30	4.48 ± 0.27	9.08 ± 0.74
EEHB 50 mg	0.38 ± 0.12	2.75 ± 0.12	6.43 ± 0.49	8.70 ± 0.33
EEHB 100 mg	0.50 ± 0.14	3.55 ± 1.10	5.48 ± 1.12	9.05 ± 0.55
EEHB 200 mg	0.68± 0.18	2.68 ± 0.48	5.48 ± 0.62	8.58 ± 0.43
EEHB 400 mg	0.63 ± 0.12	2.50 ± 0.29	5.78 ± 0.59	9.00 ± 0.62
EEHB 800 mg	0.45 ± 0.12	2.48 ± 0.35	6.20 ± 0.61	9.08 ± 0.23

Normality testing shows a significant value> α = 0.05, defines distributed data normally and does not show a significant difference between three groups without being given different doses. This means that the animals tested have the same physiological conditions, so the uric acid levels are also standard. The results are listed in Figure 2.

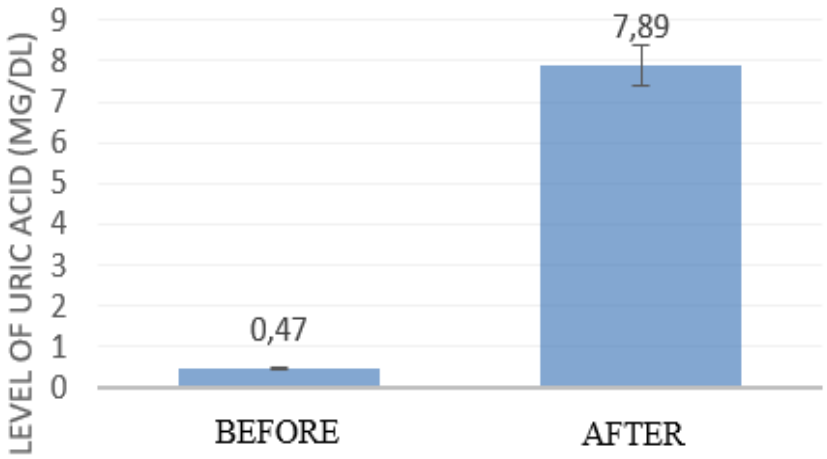

Fig 2. Uric acid levels in fasting conditions and after being induced (mean ± SEM, n = 4) Description: Data is presented in the form of an average ± SEM analyzed with paired sample test, p <0,000

Figure 2 shows that after being induced, uric acid levels rise exceed ordinary (> 4.37 ± 1.11 mg/dl) when compared before induction (fasting). Statistical testing with paired t-test results in a clear difference in uric acid levels before and after induction (p <0.05). The induction of oxonate potassium has a significant effect in increasing uric acid levels in the animals tested. Oxonate potassium is one of the fastest inducements of hyperuricemia providing hyperuricemia effects on rodents [19].

EEHB Evaluation of Rat Uric Acid Levels

After hyperuricemia, mice are given EEHB treatment for 14 days orally. Gout data (mg/dL) Each group is calculated averagely and then analyzed using ANOVA with a post hoc Tukey HSD to distinguish different treatments. The results of the decrease in uric acid are listed in Figure 3.

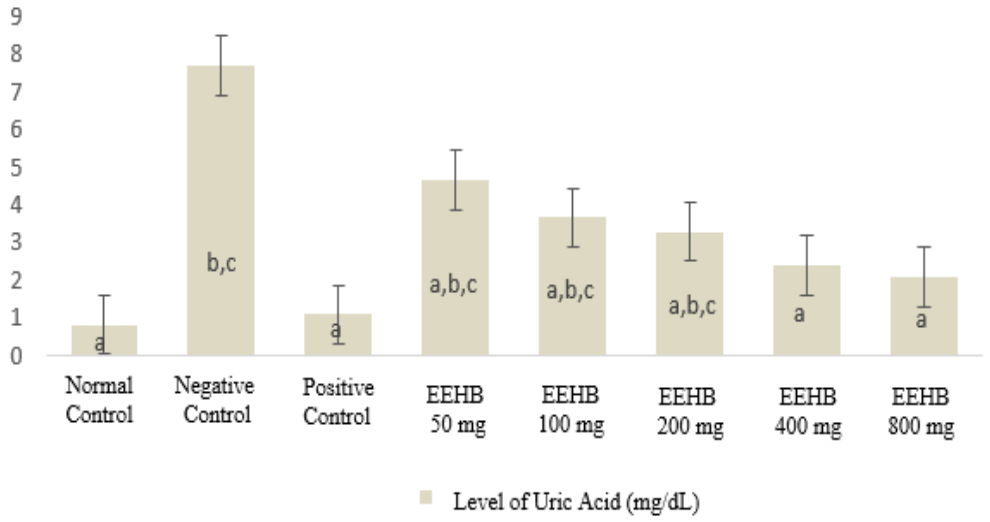

Fig 3. Result of Gout Decrease After Treatment EEHB

Note: Data is presented in the form of mean ± SEM, [a]shows the difference with the negative control, [b]shows the difference with the positive control, [c]shows the difference with normal

Statistical tests showed that the uric acid values among the normal, positive control, and EEHB administration differed significantly from the negative group, $p<0.05$. This shows that the administration of EEHB suspension affects reducing uric acid levels. The group with EEHB 400 and 800 suspensions did not have a significant difference in $p>0.05$ compared to the positive control group. These results indicated that both the EEHB 400 and 800 groups had the same level of effectiveness as the positive control. The active chemical compounds of flavonoids contained in the extract have a synergistic effect.The treatment and positive control groups showed decreased uric acid levels compared to the negative control. The standard group gave normal uric acid levels (0.8 mg/dL). The most significant reduction in uric acid levels was in the positive control (1.08 mg/dL), followed by the EEHB 800 group (2.08 mg/dL), the 400 group (2.38 mg/dL), the 200 group (3.28 mg/dL). dL), group 100 (3.65 mg/dL), and group 50 (4.65 mg/dL). The negative control group raised uric acid levels, namely 7.68 mg/dL. The standard group showed a significant difference ($p<.0.05$) from the negative group. The EEHB group at doses of 50, 100, 200, 400, and 800 did not show a significant difference ($p>0.05$) from the positive group, which was not given any treatment. This group was given enough food and drank to stabilize the synthesis and excretion of uric acid.

This group is helpful as a reference for comparing fluctuations in uric acid levels. The negative group (Na-CMC 0.5%) was still hyperuricemia, namely 7.68 ± 0.75, which showed a significant difference ($p <0.05$) with the positive group and the normal group. When uric acid levels are very high in the plasma and are filtered in large quantities, the pH of the proximal tubule changes to acid (pH 5.0), resulting in uric acid deposition in the proximal tubule and interfering with kidney function [20].The positive group compares how effective EEHB is at stabilizing uric acid levels. The positive group lowered uric acid levels compared to EEHB, namely 1.08 ± 0.47 mg/dL, giving significantly different results ($p <0.05$) from the negative group. Meanwhile, the EEHB 50, 100, and 200 groups yielded no significantly different results ($p>0.05$) from the normal, EEHB 400, and EEHB 800 groups. This is because the structure of allopurinol can occupy the active site of the XOD and irreversibly inhibits enzyme activity. This activation can inhibit the synthesis of uric acid and increase the secretion of uric acid in the urine [21].After the uric acid levels were measured in the rats, a statistical test was carried out for differences in uric acid levels before and after treatment. The normality test gives a significant value $> \alpha = 0.05$, which means that the data is normally distributed and there is no significant difference between the comparison, test and control groups. The results of measurements of uric acid levels before and after treatment are shown in Figure 4.

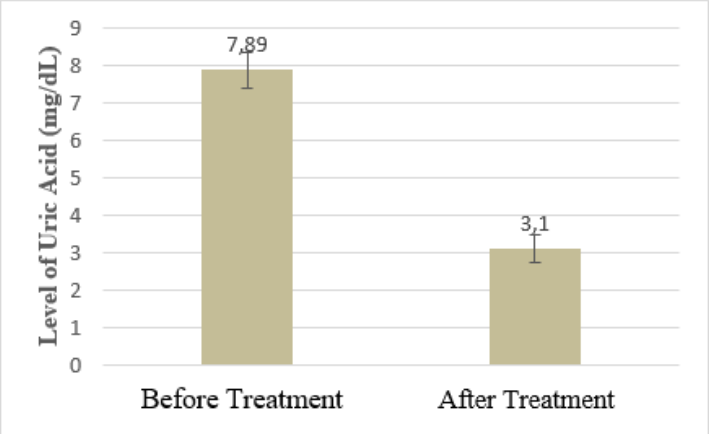

Fig 4. Uric acid levels before and after treatment (Mean ± SEM)

Note: Data is presented in the form of mean ± SEM analyzed by paired sample test (p <0.000)

The data in Figure 4 were obtained after the treatment; uric acid levels decreased by an average of 3.1 mg/dL compared to 7.89 mg/dL before treatment. Tests using the paired T-Test produced a significant difference in uric acid levels before and after treatment (p <0.05). This difference indicates that each treatment impacts stabilizing uric acid levels.

IV. CONCLUSION

The research that has been carried out produces data that confirms that Binara Herb fulfils the simplicia characterization requirements. Binara herbs contain secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, saponins and steroids. Binara Herb Ethanol Extract has been shown to reduce uric acid levels in hyperuricemia rats, with the best concentrations being 400 and 800 mg/kg BW. This is based on significantly different results p>0.05 compared to the positive control group.

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